I. SUMMARY

Etridiazole, 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) and its metabolite, 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) are extracted from water using a DVB C-18 SPE disk. The Etridiazole and dichloro-etridiazole are analyzed directly by GC using a nitrogen phosphorous detector. 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) is extracted from the water using a SAX SPE disk. The Etridiazole acid is converted to the Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate (T-28) using 1% sulfuric acid in ethanol. It is then analyzed by GC, using a nitrogen phosphorous detector.

II. MATERIALS

A. EQUIPMENT

1. Balance, analytical , Model AT 261 Delta, Mettler™
2. Balance, Model 1602 MP8-1, Sartorius
3. Vortex mixer, Fisher Scientific
4. Centrifuge, Model 228, Fisher Scientific
5. Vacuum manifold for SPE disks, 12 port, Supelco
6. Vacuum pump, 1/6 HP, Model 0211-U45N-G8CX, Gast®
7. Analytical Evaporator, Model "N-Evap", Organomation Associates, Inc. The evaporator is connected to house nitrogen line fitted with a filter and a pressure regulator, Balstone Filter Products
8. A pH Meter, Accumet™ 910, Fisher Scientific
9. Magnetic stirrer, Corning™
10. Gas Chromatograph, Hewlett Packard™ 6890+, equipped with a nitrogen phosphorous detector and an autosampler-injector Model HP 7683.
11. Computer with ChemStation HP 3365 on Windows 95 platform controls data capture
12. Finnigan MAT model TSQ 7000 equipped with a Varian™ 3400 gas chromatograph

Note: Equivalent equipment from other sources can be employed.

B. SUPPLIES

1. Centrifuge tubes, 15 mL graduated, conical-bottom with screw caps, Fisher Scientific
2. Kolmer evaporation tubes, 10 mL graduated, Fisher Scientific
3. Flasks, 1 L volumetric, Fisher Scientific

4. Filtration suction flask, 1 L, Fisher Scientific
5. DVB C-18 and SAX 50 mm solid phase extraction disks and reservoirs, Bakerbond Speedisk™, J.T. Baker®
6. Assorted volumetric disposable pipettes, Fisher Scientific
7. Customary analytical laboratory glassware and supplies

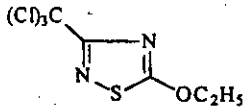
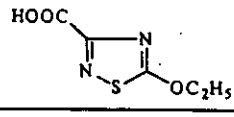
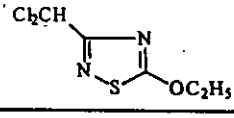
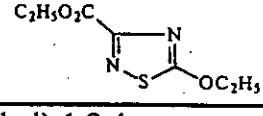
Note: Equivalent supplies from other sources can be employed. It is recommended, however, that SPE disks from J.T. Baker® be used.

C. CHEMICALS AND REAGENTS

1. Hexanes, GC Resolv™ grade, Fisher Scientific
2. Methanol, HPLC grade, Fisher Scientific
3. Methylene Chloride, Optima™ grade, Fisher Scientific
4. Water, HPLC grade, Fisher Scientific
5. Absolute Anhydrous Ethanol, AAPER Alcohol and Chemical Co. and Pharmco
6. Potassium Phosphate, J.T. Baker®
7. Sodium Hydroxide pellets, J.T. Baker®
8. Sulfuric Acid, J.T. Baker®

Note: Chemicals and reagents of equivalent purity from other sources can be employed.

D. STANDARDS-TEST SUBSTANCES

Standard	Uniroyal Chemical Co. Code	Source	CAS No.	Purity %	Molecular Weight	Structure
5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole	T-01	Olin Corp.	2593-15-9	99.6	247.52	
5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid	T-02	Uniroyal Chemical Co.	67472-43-9	100	174.17	
5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole	T-03	Uniroyal Chemical Co.	Not available	99.75	213.08	
Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate	T-28	Uniroyal Chemical Co.	Not available	100	202.2	

Note: All standards are stored in the freezer, except 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole, which is stored, refrigerated. Stability of the standards is expressed as the expiration date as stated on the COA. COA's are in Appendix II.

E. SAFETY AND HEALTH

This method should be preformed by trained chemical personnel. Hazards associated with the chemicals used in this analytical method are shown in the MSDS sheets in Appendix III.

III. ANALYTICAL METHOD

A. PRINCIPLE OF THE METHOD

The water sample is passed through a DVB C-18 Speedisk™ under vacuum. The Etridiazole and 3-dichloro metabolite, which are retained on the disk, are eluted with hexane and concentrated under nitrogen. The analysis is by capillary GC with a nitrogen phosphorous detector. The quantitation is by external standard method, using a mixture of 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) and 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) standards to generate the two calibration plots.
The same water sample is then passed through a SAX Speedisk™ under vacuum. The Etridiazole acid (5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid) is eluted with 1% sulfuric acid in ethanol. The Etridiazole acid (T-02) undergoes derivatization in the concentration step under nitrogen. The analysis is by capillary GC with a nitrogen phosphorous detector. The quantitation is by external standard method, using Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate (T-28) to generate a calibration plot. A flow diagram of the analytical procedure is shown in Figure 1.

B. WATER SOURCE AND CHARACTERIZATION

This method should be applicable for water from various types of sources. Pond water from Preston Hill Pond (Uniroyal Chemical Co.), Middlebury, CT was collected for use in this study. The TOC content is 3.3 mg/mL. TOC varies from time to time. Water collected from the same site previously (August 1998), had a TOC of 8.3 mg/mL. Characterization results are located in Appendix IV.

C. PREPARATION OF REAGENTS

C-1. 1% Sulfuric Acid in Ethanol

Fill a 250 mL volumetric flask with ~200 mL of absolute ethanol. Pipette in 2.5 mL of concentrated sulfuric acid. Mix well and bring to the mark with additional absolute ethanol. Cap and store at room temperature.

C-2. Neutralization Solution

C-2a. 2 M NaOH Solution

Using a top loading balance, weigh 40 g of NaOH and dissolve in 500 mL of HPLC grade water. Cap and store at room temperature.

C-2b. Phosphate Buffer

Slowly add 204 g of KH_2PO_4 to a 2 L beaker containing 400 mL of HPLC grade water, which is being stirred with a magnetic stirrer. Add 350 mL of the 2 M NaOH solution. Continue stirring until all the crystals are dissolved. Titrate to pH 7.0 with the 2 M NaOH solution. This should require the addition of about 100 –120 mL of the 2 M NaOH solution. Bring the final volume to 1 L with HPLC water. Cap and store at room temperature.

D. PREPARATION OF THE STANDARD SOLUTIONS

D-1. T-01, T-02, and T-03 Standard Solutions for Fortification

D-1a. T-01, T-02, and T-03 Fortification Stock Solutions

On an analytical balance, weigh 50 mg of the T-01, T-02, and T-03 standards into a 50 mL volumetric flask and dilute to volume with absolute ethanol. Vortex the flask. The concentration of the stock solution is 1 mg/ mL. Correct the concentration for the purity of the standard (if necessary): mg/mL x (Standard Purity / 100).

D-1b. T-01, T-02, and T-03 Fortification Working Solution A

Pipette 1.0 mL of Fortification Stock Solution, D-1a, into 100 mL volumetric flask and dilute to volume with absolute ethanol. The concentration of the solution is 10,000 ηg/mL.

D-1c. T-01, T-02, and T-03 Fortification Working Solution B for 0.1 and 0.5 ppb Recoveries

Pipette 1.0 mL of Fortification Working Solution A, D-1b, into 100 mL volumetric flask and dilute to volume with absolute ethanol. The concentration of the solution is 100 ηg /mL.

D-2. Preparation of T-01 and T-03 Calibration Standards

D-2a. T-01 and T-03 Standard Stock Solution

On an analytical balance, weigh 50 mg of the T-01 and T-03 standard into a 50mL volumetric flask and dilute to volume with hexane. Vortex the flask. The concentration of the stock solution is 1 mg/ mL. Correct the concentration for the purity of the standard (if necessary):
mg/mL x (Standard Purity / 100).

D-2-b. T-01 and T-03 Standard Working Solution A

Pipette 1.0 mL of Standard Stock Solution, D-2-a, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 10,000 ηg /mL

D-2-c. T-01 and T-03 Standard Working Solution B

Pipette 10.0 mL of Standard Working Solution A, D-2-b, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 1000 ηg /mL.

D-2-d. T-01 and T-03 Standard Working Solution C

Pipette 25.0 mL of Standard Working Solution A, D-2-b, into a 50 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 5000 ηg /mL.
All standard solutions are kept in the freezer when not in use.

D-3. Dilutions of T-01 and T-03 Calibration Standard

D-3-a. T-01 and T-03 Calibration Standards for the 0.1 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-01 and T-03 Standard Working Solution B (D-2-c). Dilute to volume with hexane. The concentrations of the resulting standard solutions are: 50, 75, 100, 125, and 150 ηg /mL, respectively.

D-3-b. T-01 and T-03 Calibration Standards for the 0.5 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-01 and T-03 Standard Working Solution C (D-2-d). Dilute to volume

with hexane. The concentrations of the resulting standard solutions are: 250, 375, 500, 625, and 750 ηg /mL, repectively.

D-4. Preparation of T-28 Calibration Standard

D-4-a. T-28 Standard Stock Solution

On an analytical balance, weigh 50 mg of the T-28 standard into a 50 mL volumetric flask and dilute to volume with hexane. Vortex the flask. The concentration of the stock solution is 1 mg/ mL. Correct the concentration for the purity of the standard (if necessary):
mg/mL x (Standard Purity / 100).

D-4-b. T-28 Standard Working Solution A

Pipette 1.0 mL of Standard Stock Solution, D-4-a, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 10,000 ηg /mL.

D-4-c. T-28 Standard Working Solution B

Pipette 10.0 mL of Standard Working Solution A, D-4-b, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 1000 ηg /mL.

D-4-d. T-28 Standard Working Solution C

Pipette 25.0 mL of Standard Working Solution A, D-4-b, into a 50 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 5000 ηg /mL.
All standard solutions are kept in the freezer when not in use.

D-5. Dilution of T-28 Calibration Standard

D-5-a. T-28 Calibration Standards for the 0.1 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-28 Standard Working Solution B (D-4-c). Dilute to volume with hexane. The concentrations of the resulting standard solutions are: 50, 75, 100, 125, and 150 ηg /mL, respectively.

D-5-b. T-28 Calibration Standards for the 0.5 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-28 Standard Working Solution C (D-4-d). Dilute to volume with hexane. The concentrations of the resulting standard solutions are: 250, 375, 500, 625, and 750 ηg /mL, respectively.

E. FORTIFICATION OF WATER SAMPLES

E-1. Fortification with T-01, T-02, and T-03 at the 0.1 ppb level

Fill a 1 L volumetric flask with ~975 mL of water. Pipette 1 mL of Fortification Working Solution B (D-1-c) into the flask. Bring to the mark with additional pond water. Mix well, shaking by hand.

E-2. Fortification with T-01, T-02, and T-03 at the 0.5 ppb level

Fill a 1 L volumetric flask with ~975 mL of water. Pipette 5 mL of Fortification Working Solution B (D-1-c) into the flask. Bring to the mark with additional pond water. Mix well, shaking by hand.

F. EXTRACTION AND CLEANUP PROCEDURE

F-1. Extraction and cleanup of T-01 and T-03

1. Mount a DVB C-18 Speedisk™ to a SPE manifold. Wash with 20 mL of methylene chloride. Allow the disk to soak for at least 2 minutes. Apply a low house vacuum to dry the disk. Add hexane (2 X 15 mL) and dry the disk again.
2. Condition the disk with 10 mL of methanol. Allow disk to soak for 1 minute. Pull through most of the remaining methanol, leaving 3-5 mm of methanol above the surface of the disk. From this point to the end of sample addition, do not allow the disk to run dry.
3. Repeat step 2 using 10 mL of HPLC grade water.
4. Transfer the disk to a 1 L suction flask connected to a vacuum pump. Attach the reservoir/adapter to the disk housing. Add water sample under vacuum at about 15 inches of Hg. (Allow 3-5 minutes for 1 L sample.) Transfer disk back to SPE manifold and dry under full vacuum (~20 inches of Hg) for at least 30 minutes. Transfer the eluted water back to the 1 L flask to be saved overnight, at ambient temperature for the extraction of T-02.
5. Using gravity, elute the 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) and 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) with 10 mL of hexane

6. into a 10 mL Kolmer evaporation tube. Apply gentle vacuum, < 5 inches of Hg., to elute any remaining analyte.
7. Concentrate the extract under a gentle stream of nitrogen gas at ambient temperature to less than 1 mL. Bring to the 1 mL mark with hexane. Vortex gently and transfer to autosampler vial for GC-NPD analysis.

F-2. Extraction and cleanup of T-02.

1. Mount a SAX Speedisk™ to a SPE manifold. Wash with 20 mL of 1 % H_2SO_4 in ethanol. Allow the disk to soak for at least 2 minutes. Apply a low house vacuum to dry the disk.
2. Condition the disk with 10 mL of methanol. Allow disk to soak for 1 minute. Pull through most of the remaining methanol, leaving 3-5 mm of methanol above the surface of the disk. From this point to the end of sample addition, do not allow the disk to run dry.
3. Repeat step 2 using 10 mL of HPLC grade water.
4. Transfer the disk to a 1 L suction flask connected to a vacuum pump. Attach the reservoir/adapter to the disk housing. Add water sample from F-1, step 4 (pg. 17) under vacuum at 15 inches of Hg. (Allow 3-5 minutes for 1 L sample.) Transfer disk back to SPE manifold and dry under full vacuum (~20 inches of Hg) for at least 30 minutes. Discard the eluted water .
5. Using gravity, elute 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) with 10 mL of 1 % H_2SO_4 in ethanol into a 15 mL graduated conical centrifuge tube . Apply gentle vacuum, < 5 inches of Hg., to elute any remaining analyte.

G. DERIVATIZATION OF T-02 WITH 1 % H_2SO_4 IN ETHANOL

1. Concentrate and derivatize the sample from F-2, step 5, by immersing the conical centrifuge tube to the 10 mL mark in a 50°C (±1°C) water bath and evaporating the solvent under a gentle stream of nitrogen gas to a final volume of 2 mL. This takes about 30-45 minutes.
2. Cool the sample to room temperature and add 5 mL of neutralization solution (phosphate buffer). Vortex at high speed for 30 seconds.
3. Add 5 mL of hexane to sample and vortex for 1 minute at high speed.
4. Centrifuge the sample for 2 minutes using a single speed centrifuge at about 3300 rpm.
5. Transfer 4.5 mL of the hexane layer to a 10 mL Kolmer evaporation tube.
6. Repeat steps 3 and 4.
7. Transfer the second 4.5 mL of the hexane layer to the 10 mL Kolmer evaporation tube. The final volume of hexane extract should be exactly 9 mL. The extract contains Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate (T-28).

8. Concentrate the extract under a gentle stream of nitrogen gas at ambient temperature to less than 1 mL. Bring to 1 mL mark with hexane. Vortex gently and transfer to autosampler vial for GC-NPD analysis.

H. GC-INSTRUMENTATION

H-1. Description

Gas Chromatograph, Hewlett Packard, Model HP 6890+, equipped with a nitrogen phosphorous detector and an autosampler-injector Model HP 7683.

Instrument parameter settings and a computer with ChemStation HP 3365 on Windows 95 platform controls data capture.

H-2. Conditions

Column Type: J & W DB-17, 30m, 0.25µm, 0.25 Film Thickness Model #: 122-1732				
NPD Detector *: Hydrogen: 3.0 mL/min Air: 60 mL/min Constant column & Makeup 10 mL/min Makeup gas Helium Adjust offset 35 Temp. 325°C				
Injector: Splitless Temp: 210°C Pressure: 25 psi Purge Time: 2.00 min Purge flow: 60.0 mL/min Liner: Cyclosplitter Gooseneck				
Carrier gas: Helium, at 2.7 mL/min				
Oven Temp Program:	Initial temperature: 50°C Initial Time: 2.00 min			
	Ramp #	Rate °C/min	Final Temperature °C	Final Time min
	1	30.0	100	0
	2	10.0	180	0.5
	3	15.0	220	0
	4	50.0	270	7
Final Run Time 22.83 min				

**Note: NPD beads have a very short life.*

H-3. Calibration and Injection Sequence

1. Start the sequence by injecting the solvent blank and then the standards, starting with the lowest concentration.
2. Inject a solvent blank and then a reagent blank followed by the control samples.
3. Inject fortified controls and then the samples.
4. Complete the sequence by injecting the standards, starting with the lowest concentration.

I. POTENTIAL INTEFERENCES

This method could have potential interference from other halogenated pesticides that might elute with similar retention times.
Contamination from SPE disks can cause interference, therefore washing the disk as described in Section F-1 and F-2 is very important. A confirmatory technique should be used as required by the guidelines, if a problem is suspected (see section J).

J. CONFIRMATORY TECHNIQUE

The method for the identification of T-01, T-03, and T-02 as T-28 was by GC/MS/MS with chemical ionization(CI). Confirmation of the identity of T-01, T-03, and T-02 as T-28 was made by the comparison of the mass spectra of the peaks in the extract isolated from the 0.1 ppb fortified pond water with the spectra of the standards. The molecular ions for T-01, T-03, and T-28 are 247 and 213, and 202, respectively. The daughter ions of m/z 247 are 218 and 182 m/z. The daughter ions of m/z 213 are 184 and 148 m/z. The daughter ions of m/z 202 are 174, 146,and 128 m/z. Chromatograms and spectra are in Appendix V.

K. TIME REQUIRED FOR ANALYSIS

The time required to complete the extraction and concentration of T-01 and T-03 is about five hours. The time required to complete the extraction, derivatization and concentration of T-28 is about six hours. The GC-NPD analysis is usually carried out overnight,using an autosampler.

L. MODIFICATIONS OF POTENTIAL PROBLEMS

None.

M. METHODS OF CALCULATION

M-1. Calibration Plot

Peak area counts of the standard (T-01, T-03, or T-28) is the dependent variable and the concentration of the standard solution, expressed as ηg /mL, is the independent variable which are employed to generate a linear regression equation to determine the intercept, the slope and the linearity of detector response (coefficient of determination, R^2).

$$Pk.area = Intercept + Slope\ x\ (\eta g\ /mL)_{Std.}$$

M-2. Calculate the amount of T-01 or T-03 in the Hexane Extract

Using the peak area of T-01 or T-03 found in the extract, the concentration is determined using the following equation:

$$T-01\,(\eta g/mL) = \frac{Pk.area_{\ sample} - Intercept}{Slope}$$

If peaks are found in the control samples. A corrected peak area value is determined using the following formula:

Peak area $_{sample}$ corrected = Peak area $_{sample}$ - Peak area $_{control}$

M-3. Calculate the amount of T-02 Equivalents in the Hexane Extract

Using the peak area of T-28 in the analyte calculate the concentration of T-02 equivalents in the hexane extract and multiply this by the ratio of molecular weights of the two standards (T-02/T-28) and also multiply by the volume correction (10/9mL,see Section G, steps 5,6 & 7).

$$T-02\,(\eta g/mL) = \frac{Pk.area_{sample} - Intercept}{Slope} X \frac{174}{202} X \frac{10}{9}$$

M-4. Calculate the amount (ppb) of T-01, T-02, or T-03 found in the sample

Divide the amount of analyte calculated in Section M-2 or M-3 by the sample volume, expressed in mL.

$$ppb = \frac{\eta g / mL}{mL}$$

M-5. Calculate % Recovery of T-01, T-02, or T-03 from Fortified Control

Divide the value calculated in Section M-2 or M-3, which is the amount of analyte found in the extract, by the amount of analyte added to the control sample and multiply this value by 100.

$$\%\,\mathrm{Recovery} = \frac{\eta g\ Found}{\eta g\ Added} X\,100$$

M-6. Calculate the 95% Confidence Limits

$$CL = \frac{t \times SD}{\sqrt{n}}$$

Where SD = standard deviation

n = the number of observations

t = the value t for n-1 degrees of freedom at 95% confidence as taken from the table C.3, page 267 of Quality Assurance of Chemical Measurements, John K. Taylor, Lewis Publishers Inc. 1987.